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Philosophical Transactions of the Royal Society A

Title: Control of cell behaviour through nanovibrational stimulation - nanokicking

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Abstract

Mechanical signals are ubiquitous in our everyday life and the process of converting these mechanical signals into a biological signalling response is known as mechanotransduction. Our understanding of mechanotransduction, and its contribution to vital cellular responses, is a rapidly expanding field of research involving complex processes that are still not clearly understood. The use of mechanical vibration as a stimulus of mechanotransduction, including variation of frequency and amplitude, allows an alternative method to control specific cell behaviour without chemical stimulation (e.g. growth factors). Chemical-independent control of cell behaviour could be highly advantageous for fields including drug discovery and clinical tissue engineering. In this review a novel technique is described based on nanoscale sinusoidal vibration. Using finite element analysis in conjunction with laser interferometry, techniques that are used within the field of gravitational wave detection, optimisation of apparatus design and calibration of vibration application has been performed. We further discuss the application of nanovibrational stimulation, or 'nanokicking', to eukaryotic and prokaryotic cells including the differentiation of mesenchymal stem cells towards an osteoblast cell lineage. Mechanotransductive mechanisms are discussed including mediation through the Rho-A kinase signalling pathway. Optimisation of this technique was first performed in 2D culture using a simple vibration platform with an optimal frequency and amplitude of 1 kHz and 22 nm. A novel bioreactor was developed to scale-up cell production with recent research demonstrating that mesenchymal stem cell differentiation can be efficiently triggered in soft gel constructs. This important step provides first evidence that clinically relevant ("3D") volumes of osteoblasts can be produced for the purpose of bone grafting, without complex scaffolds and/or chemical induction. Initial findings have shown that nanovibrational stimulation can also reduce biofilm formation in a number of clinically relevant bacteria. This demonstrates additional utility of the bioreactor to investigate mechanotransduction in other fields of research.

1. Introduction

The ability for cells to sense and respond to their environment is vital for correct function and ultimately cell survival. The classical view of this process is rooted in terms of chemical signalling, as exploited through biological assays and molecular methods to elucidate signalling pathways. There has been an under appreciation of the importance that mechanical cues play in how cells sense their local environment and trigger signalling (termed mechanotransduction) [1]. This has been a focus of research in recent decades and it is now known that eukaryotic cells have evolved to respond to a plethora of mechanical stimuli (both internal and external to the cell) and physical cues experienced in daily life [2].

The ability to convert mechanical signals into a biological response is recognised as an important mechanism in many species; in humans, it provides the abilities of proprioception, touch, hearing and balance [3, 4]. Classically, the view was held that signalling events were primarily controlled through biochemical processes *e.g.* enzyme activity and reaction rates. However, there is growing evidence that the physical micro- and nano-environment is critical for the correct functioning and survival of many eukaryotic cells. This is best demonstrated in the absence of mechanical stimuli or through alterations of mechanosensitive genes and proteins. Such mutations have been implicated in the pathology of a number of disease states such as: atherosclerosis [5], deafness [6], pathobiology of bones [7], muscular dystrophy [8], and tumours [9]. For example, tumour tissue can be partially diagnosed by assessing increased tissue stiffness, and evidence of changed mechanotransduction response at the single cell level. The mechanotransductive response of cancer cells is type-dependent, with both reduction of the mechanotransduction response [10, 11] and activation of mechanotransduction pathways that promote tumour progression [12]. Changes in tissue stiffness are also implicated in fibrotic lung disease and the inability to respond to the local microenvironment [13].

An underpinning example of mechanotransductive mechanism is the ability of the mammalian cell cytoskeleton to respond to local physical cues, such as the rigidity of the microenvironment. Exogenous forces are transmitted to cells via the local environment stiffness. The elastic modulus of a material gives an indication of its

stiffness, with stiff materials having a high elastic modulus. The human body has a distinct range of elastic moduli from fat at 0.5 -1 kPa (soft) through to bone (hard) at 15 – 20 GPa [14]. Mammalian cells can also sense the force generated by fluid shear stress as demonstrated in flow cell models [15-17] and force exerted due to gravity [18]. These brief examples illustrate the diverse nature of forces both internal and external that cells sense and respond to. Unravelling the complex mechanisms of mechanotransduction has been aided by the invention of instrumentation and methods able to deliver external mechanical stimuli to individual or multiple cells in culture, for example atomic force microscopy [19] and optical tweezers [20].

This review article provides an overview of mechanotransductive mechanisms based on current experimental studies. Nanoscale vibration is taken as an example to illustrate mechanotransductive pathways along with the associated impact on mesenchymal stem cell (MSC) osteogenesis and bacterial biofilm formation. The key importance of precision measurement and computer aided design optimisation is highlighted in the development of the bespoke nanovibrational bioreactor system used by these studies. Consistency of vibration, being critical to biological reproducibility, is achieved through use of these techniques.

2. Cellular response to mechanical stimulus

The sensitivity of cells to their mechanical environment relies on three processes. Firstly, there must be a source of applied force, either externally applied (e.g. hydrostatic pressure, shear flow, gravity) or applied by the cell itself through cytoskeletal contractility. This force must then impose on specific, mechanically sensitive, proteins such as ECM binding proteins, stretch-sensitive ion channels (e.g. through deformation of the membrane) or the cytoskeleton as a whole. Many proteins are capable of conformational changes or protein folding in response to typical forces imposed by the cellular environment [2]. Conformational change allows new phosphorylation reactions to occur or ion/protein influx in the case of channel proteins leading to initiation of intra cellular signalling [21, 22]. Finally, altered signalling can yield changes in cell behaviour following signal transmission to the nucleus. Signal propagation methods include molecular translocation, diffusion and even stress wave propagation directly to the nucleus through the cytoskeletal network [23]. Of these

three mechanisms, stress wave propagation can occur rapidly, within timescales of around 1 ms [23] via protein transducers such as membrane spanning focal adhesion complexes connected to integrins [24]. Studies comparing chemical and mechanical stimulation have demonstrated that the latter can yield significantly faster activation of specific kinases (>12 s vs <300 ms respectively) and this colocalised with areas of cytoskeleton deformation [25].

Focal adhesions demonstrate a key example of tension based conformational change, with each complex made of multiple subunits forming the overall mechanotransducer. Actin linking and polymerising proteins include proteins such as vinculin and talin which connect the cytoskeleton to ECM bound integrins however it is the signalling subunit within the focal adhesion complex provides force based modulation of actin polymerisation and the actin/myosin contractility process [26]. Proteins such as focal adhesion kinase (FAK) mediate this signalling by allowing additional phosphorylation to occur following mechanical conformational change leading to downstream signalling [27].

Integrins provide the anchorage which is key to producing increased cytoskeletal tension. Mechanical manipulation of integrins using microbeads demonstrates the entire system in action with axial rearrangement of the cytoskeleton and deformation of the nucleus [28], both effects which are not seen when manipulating other membrane bound proteins [29]. Of course, there are a family of integrins with a high degree of specificity in their connection to the cytoskeleton. For example, studies have linked integrin $\alpha3\beta1$ with actin filaments and integrin $\alpha6\beta4$ to intermediate filaments [30].

Another example of force based protein manipulation are stretch activated ion channels with a number of channels being gated specifically by mechanical force [31]. Opening of these channels creates an ion gradient flow [32, 33] which causes diffusion into the cytoplasm and thus possible interaction with various biochemical pathways. In *Escherichia coli* (*E. coli*) mini, small and large mechanically gated channels play a role adaption to osmosis [33, 34]. There are many examples in eukaryotic cells including sensory neurons converting the sense of touch to action potentials [35] and in aortic endothelial cells transient receptor potential (TRP) channels are involved with

modulation of Ca^{2+} influx when cells are under tension [36]. The specific mechanism of this has been probed through techniques such as patch clamping however there is still debate if the gating process is reliant on membrane tension from underlying cytoskeletal proteins (e.g. spectrin) [37] or whether lipid bilayer tension alone is sufficient to activate these channels [38].

Completing the structural picture, it is also important to consider the direct mechanical integration of the ECM to the nucleus via linker of nucleoskeleton and cytoskeleton (LINC) complexes [39]. In a similar manner to the cellular membrane, stress can be directly transmitted to the nuclear envelope, resident ion channels [40] and even the chromatin itself with force based changes in conformation being possible [41, 42]. This was analytically calculated for nanoscale vibration by Curtis *et al.* [43].

These processes can be summarised using two models; the *switch-like model* and the *dynamic model* [21]. The switch-like model is a basic system which describes the progression of a mechanical signal and how the cell senses and responds to it. This system is summarised in **Figure 1A** and shows the process of transmission (mechanotransmission) of the mechanical cue, followed by the sensing of this cue by the cell (mechanosensing) resulting in a biological response (mechanoresponse). Mechanotransmission describes the transmission of the force from adhesion proteins through the cytoskeleton structures e.g. actin [44], microfilaments [45], microtubules [46] and intermediate filaments [47]. These structures allow forces to travel away from the initial exertion point and propagate along the cell cytoskeleton. As a consequence of the propagation of the force, mechanosensing occurs due to protein conformational changes. It is also important to note that mechanoresponse can describe rapid downstream molecular pathways but may also play a role in long term response e.g. arterial wall thickening and bone remodelling [5]. Microgravity is a prime example of altered bone remodelling which has been shown to induce osteopenia [48, 49], a loss of bone density.

The switch-like model of mechanotransduction adequately describes the basic molecular diffusion processes in mechanotransduction, however a dynamic model is required to describe the mechanosensation in force wave propagation as the switch-like model is not appropriate as it fails to account for time dependant application of

force *e.g.* continuous stretch versus cyclic stretch [50, 51], and the frequency of the stimulus [52]. Specific frequencies have also been observed to elicit different cellular responses. The hierarchical mechanotransduction response of switch-like models forms a basis to understanding the process but it is apparent that dynamic elements need to be accounted for.

Dynamic mechanotransmission details the transmission of force through the cytoskeleton of the cell taking into consideration the constant remodelling of the load bearing structures and the interactions between those different components *e.g.* actin – myosin bonds. These adaptive properties of the cytoskeleton can alter local viscoelasticity due to reinforcement of certain structures or fluidisation of others (**Figure 1B**) [53]. Reinforcement of the cytoskeleton is classically noted in MSC derived osteoblasts with increased actin stiffening and contractility of the cell when cultured on nanotopographies [54], in response to stiff materials [55], and if spreading is induced through increased cell-adhesion ligation. In contrast, fluidisation results in a disruption of the cytoskeletal structures in specific areas. The combination of reinforcement and fluidisation thereby allows continued transmission of these forces in a directed manner, where transmission will occur along the reinforced network, but upon reaching regions where fluidisation has occurred, will be dampened [56].

There is strong evidence to suggest that the cytoskeleton can act as a bandgap filter meaning that the cell is only responsive to certain frequencies [57]. The cytoskeleton is composed of linker molecules that have different elastic constants, altering the cellular ratio of these linkers may allow the ‘filter’ to be tuneable [58]. This is further evidenced by force propagation over longer intra-cellular distances being controlled by the contractile pre-stress of the cytoskeleton and the loading frequency [59]. Experimental evidence also suggests that these frequencies are species specific as shown in the auditory transduction of mammals (rat) occurring faster than reptiles (turtle) due to the larger auditory frequency range of mammals [60]. This suggests that dynamic mechanosensing is strongly interlinked with dynamic mechanotransmission when considering the transmission of the mechanical force along the load bearing structures. As noted, these load bearing structures are governed by the ratio of the bonds between them and these bonds are also susceptible to being broken when transmitting a mechanical force. Experimental data suggests that forces in the order

of hundreds of piconewtons can break single actin fibres with fibre bundles requiring much larger breaking forces of 300 to 600 nanonewtons [61, 62] (**Figure 1C**). Once a bond is broken, transmission of the mechanical force through these two previously bonded structures halts. Dynamic mechanotransduction can also be influenced by the downstream mechanoreponse of the biological pathways involved with the regulation of the cytoskeleton and adhesion structures, effectively acting as a ‘feedback’ loop to enhance or diminish mechanotransduction (**Figure 1D**).

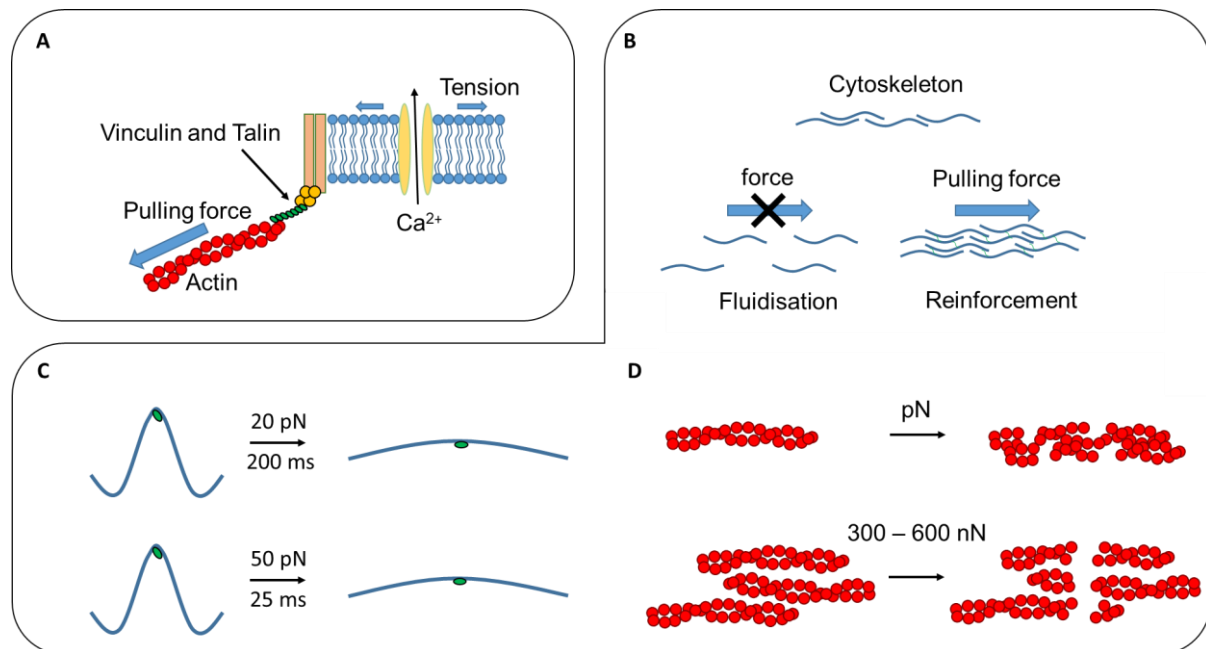


Figure 1 – Simplified schematics of switch-like and dynamic elements of mechanotransduction

(A) schematic representation of a common mechanism of mechanotransduction. Conformational change due to tension exerted on membrane results in the opening of ion channels. This allows a flow of ions across the membrane which triggers specific signalling pathways. The mechanotransduction response is dynamic with factors such as frequency, static versus cyclic stretch and duration of application influencing dynamic mechanotransduction. (B) Transmission of force can be directed through the cellular cytoskeleton by a combination of fluidisation (stop transmission) or reinforcement (enhance transmission) [56]. (C) The magnitude of force applied to a protein filament has been shown to dictate the rate of conformational change. Forces and rates shown were experimentally derived by Del Rio *et al.* [63]. (D) Reinforced structures are more resistant to breaking; reinforcement of the actin structures, for example, can increase the breaking force by a factor of up to 10 000 [61, 62]. The transmission of a force along the structure can also aid in the reinforcement of the structure in a positive feedback loop.

3. Measurement at the nanoscale

Producing accurate mechanical signals that are transmitted to the cells can be achieved using piezoelectric materials, or piezo actuators. Applying a constant electric

field to a piezo ceramic deforms its shape, therefore, applying a time varying electric field, such as a sine wave, can create a source of vibration. In nanovibrational studies, a well-defined sine wave is chosen allowing the peak acceleration, and therefore peak force, experienced by the cells to be estimated [43]. Other wave forms (e.g. square waves) have not been studied due to uncertainty in the acceleration and force associated with the corners of the waveform.

Initially the nanovibrational studies were carried out in small petri dishes attached to single piezo actuators [43, 64, 65]. However, the process was scaled-up to apply vibration across standard multi-well and flask culturewares [66, 67]. The bioreactor design currently consists of an array of piezo actuators sandwiched between a heavy aluminium base and a top plate. The top plate comprises an aluminium and a magnetic stainless steel plate, configured such that the aluminium side is against the array of piezos and the stainless steel plate sits on top allowing cultureware to be magnetically attached.

Nanoscale displacements produced by the bioreactor are measured using laser interferometry, which is used in a diverse range of research areas, including gravitational wave astronomy, where it has been used to make the first direct detection of gravitational waves [68-71]. Gravitational wave interferometers are able to measure displacements of the order 10^{-20} m over a distance of 4 km [68]. In the biological studies reviewed in this paper, a table-top laser interferometer (Model ST-S 120, SIOS Meßtechnik GmbH, Ilmenau, Germany), is used (**Figure 2**), and is capable of resolving displacements of 0.1 nm. Displacement amplitudes across different cultureware types are shown in **Table 1**. Estimation of the accelerative force applied to single cells has previously been calculated using assumptions of the fluid mass being moved during vibration (the mass being accelerated) and found to be in the nN range [43].

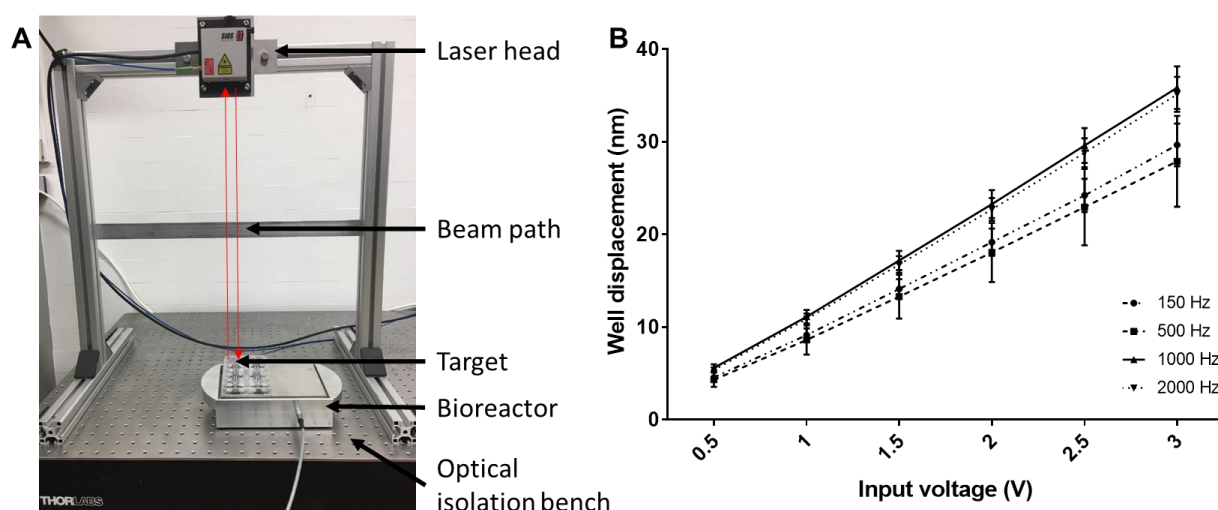


Figure 2: Precision measurement of nanoscale displacements

(A) Measurement of nanoscale displacements are performed using a laser interferometry. (B) Calibration of 6 well tissue culture plates on bioreactor shows a linear correlation between bioreactor input voltage and vibration displacements generated across a range of frequencies.

Table 1.

Measurement of vibration displacement produced in a range of commonly used tissue cultureware at 1 kHz. Measurements were performed for each well of the 6 and 12 well plates and at 9 and 21 points on the bottom surface of the T75 and T150 flasks respectively. Data are mean \pm standard deviation (Stdev)

	Average displacement (nm)	Stdev (nm)
6 well	29.6	± 1.9
12 well	31.2	± 2.3
T75 flask	31.8	± 2.0
T150 flask	33.2	± 2.6

FEA modelling has been critical in the design of the bioreactor to predict and correct for the effect of vibrational resonance in the device and cultureware. A modal analysis can provide information on exactly how the chosen cultureware will deform at each of its resonant modes. For example, it was shown in [65] that a 52 mm petri dish has a resonant mode at 339 Hz when mechanically stimulated by a single piezo at the centre of the base of the dish. The saddle shape of this mode would result in cells near the edge of the dish receiving almost double the vibration amplitude than the cells at the centre of the dish, leading to inconsistent cell stimulation. This analysis highlights that all cultureware will have an upper frequency limit for the production of consistently stimulated cells which could be significant not only for research based experiments, but also for future medical trials where reproducibility and consistency are crucial.

Suitable modification of the cultureware (increasing rigidity) can help increase the first internal resonance and thus extend the frequency range where the system behaves as a rigid body. In addition, harmonic analysis can be used to inform the design of the bioreactor and cultureware, e.g. placement of piezos and material selection, predicting the nanoscale displacements transmitted to the cultureware and thus cells. An example of the bioreactor top plate is shown in **Figure 3**, where a subtle difference in the predicted nanoscale displacements are shown when comparing a 1 and 2 kHz frequency.

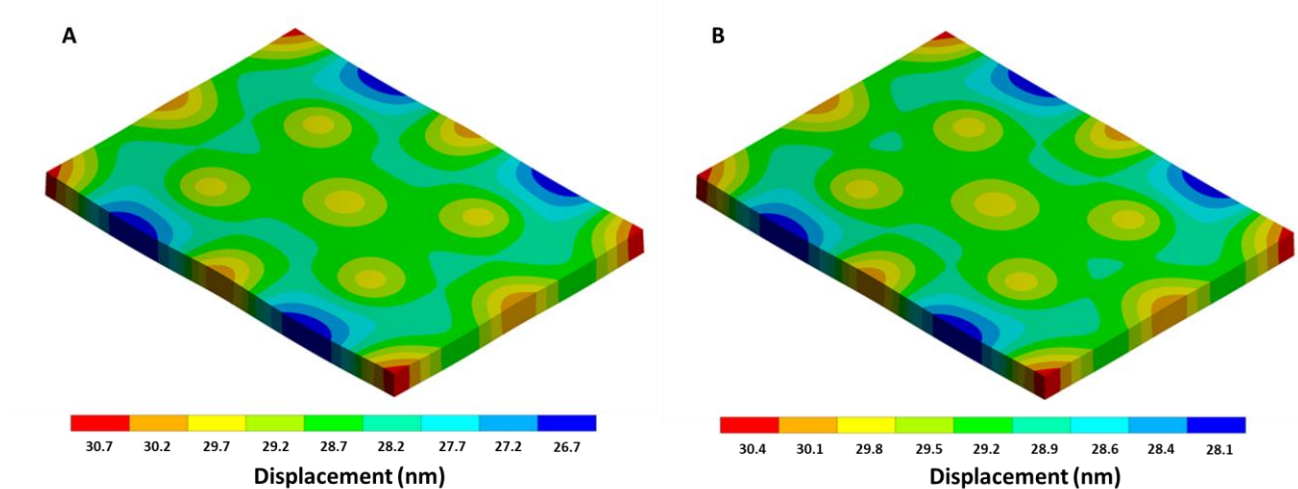


Figure 3: Harmonic analysis of bioreactor top plate

Modelling of the harmonic response of the bioreactor top plate can be performed to better understand the bioreactor behaviour. (A) Harmonic response of top plate at 1 kHz frequency. (B) Harmonic response of top plate at 2 kHz. Initial 30 nm displacement applied to underside of top plate to simulate the action of the 13 piezos. To simulate gravitational loading, an acceleration of 9.806 ms^{-1} was applied in the opposite direction of piezo action.

4. MSC cell differentiation and mechanical stimulation

Bone is the second most transplanted tissue in humans and is commonly grafted from the iliac crest (donor site) to the recipient site [72]. The volume of autologous bone which can be removed this way is limited and can often be associated with chronic donor site pain, post-operative infection and other donor-site morbidities [73]. Bone undergoes modelling and remodelling in response to physical external factors to maintain structural strength and mineral homeostasis. This remodelling is controlled through the actions of osteoblasts and osteoclasts, bone building and bone resorbing cells respectively. Imbalances in the endosteal resorption of bone and periosteal

apposition may lead to conditions such as osteoporosis [74]. MSCs are multipotent stromal cells that differentiate into a number of cell types associated with the musculoskeletal system such as osteoblasts, adipocytes, chondrocytes and fibroblasts [75]. MSCs reside in virtually all post-natal tissue [76, 77] but are commonly isolated from the bone marrow [78]. Adipose (fat) tissue is also rich in adipose derived MSCs (AdMSCs) [79].

Controlling MSC differentiation is therefore highly desirable to address the clinical need, as such experimental techniques have focused on passive (e.g. topographical control and environmental stiffness) and active methods (e.g. gravity, shear flow and vibration). Passive techniques focus on the generation of internal tension in the cell by altering the physical environment the cells attach too. This can be accomplished by altering the material or by topographical patterns, whereby different levels of stress can be achieved by varying the parameters [80]. Microscale patterns are a well-researched area, and effects have been shown to have a marked effect on cell behaviour and ultimately in the case of MSCs, differentiation. These effects can also occur through alteration of the surface with specific ECM proteins and/or polymers [81-83]. Addition of these components to the surface can alter the absorbed ECM protein or by changing the electrostatic potential of the surface [84]. Microscale features have been extensively investigated aided by relevant technology, e.g. photolithography, and have been found to regulate cell functions including but not limited to proliferation, differentiation and apoptosis [82-84]. With the advance of microfabrication techniques, extensive investigation of ordered microscale structures e.g., pillars [85, 86], pits [87] and groves [88, 89], was achievable and contributed greatly to the effect these structures have on cellular mechanotransduction. Microscale features in the context of implants have been noted to alter osteoblast behaviour [90, 91].

Nanoscale features known as nanotopographies have been shown to have strong impact on the morphology and phenotype of MSCs [92, 93], and alter other cell responses such as proliferation. Dalby and colleagues used a series of ordered and disordered nanoscale grids formed from pits with geometry 120 nm diameter and 100 nm deep [92]. While the ordered nanoscale grids showed minimal osteogenesis, the disordered near square pattern showed the largest increase compared to the hexagonal and perfect square. A further study, altering the height of titania nanopillars,

demonstrated an inverse relationship between osteoinductive effect and feature height (15 nm being optimal) [94]. Reduction of the nanopillars to 8 nm diminished this effect suggesting that there was a critical cut off size for cell filopodia interaction with nanofeatures. These experiments also revealed fine nanoscale projections, promoted by the 8 nm features, which are now termed 'nanopodia'. Additional investigation revealed large changes in adhesion, nucleus and lamin morphologies leading to the suggestion that direct (mechanical) and indirect (biochemical) signalling are critically important in regulating stem cell fate [95]. It has also been shown that 350 nm gratings affect human MSC adhesion and migration [93]. It was noted that mature focal adhesions of a smaller size were observed and zyxin was identified as being responsible for this due to reduced intracellular tension. It was hypothesized that the 350 nm gratings showed a similar response to compliant surfaces. Thus, how MSCs adhere and spread on materials is important for subsequent differentiation. Surfaces that stimulate adhesion drive increased cytoskeletal contraction and enhanced osteogenesis [96, 97]. A classical example is from confinement of MSCs to small adhesive areas (e.g. 1000 μm^2), which restricts cell spreading and thus induces adipogenesis, while larger surface areas (e.g. 10 000 μm^2), which facilitates cell spreading, induces differentiation of MSCs towards an osteoblast lineage. This occurs due to changes in actin–myosin contraction mediated through Rho-A kinase (ROCK) [56].

In the human body the local environment in which cells reside can cover a diverse stiffness range from <kPa through to tens of GPa [14]. Mechanotransduction processes can be activated by pre-stress in the cytoskeleton in addition to the potential that alteration of the rigidity may also be sufficient to impact on cellular differentiation. MSCs have been shown to respond to the elasticity of their environment and stiffness gradients e.g. between tissue types. Hydrogels are a valuable tool for the investigation of cellular response within a “3D” matrix. These gels can be synthesised from an array of biological and synthetic polymers with the ability to modulate the elastic modulus by changing the cross-linking [98, 99]. The ability to tailor stiffness coupled with being highly hydrated has resulted in hydrogels being useful in mimicking human tissue to study cell behaviour [100, 101]. Mimicking the elasticity of 3 distinct tissue types, that of brain, muscle and bone, hydrogels seeded with MSCs showed phenotypic switches towards the cell type of the mimicked tissue type [55]. Another study demonstrated

that MSCs are capable of migrating along these gradients (1 Pa/ μm to 100 Pa/ μm) to the stiffest regions of these gradients in a process known as durotaxis [102]. In addition, their data suggested that a functional actin cytoskeleton is required to achieve this migration and microtubules are required for this migration to be directed.

Active methods such as gravity, compressive loading and shear have also been shown to be inducers of MSC differentiation. Hypergravity (10g) has been shown to increase cell proliferation in addition to up-regulation of runt-related transcription factor (RUNX2), which gives some indication of an increase in osteoblastogenesis [103]. Cyclic compressive loading has been shown to have the potential to induce chondrogenesis of rabbit bone marrow derived MSCs by synthesis of TGF-beta1 [104]. Combination of cyclic compressive loading with added TGF-beta1, showed no significant difference when compared to TGF-beta1 and cyclic compressive loading only. While shear flow is often thought of as influencing endothelial cells due to their periodic cycling of pressure and flow when exposed to blood flowing the human body [105], there is also evidence to suggest that low interstitial shear flow can induce MSCs towards an osteogenic lineage. Low sheer flow stress induces osteogenesis of MSCs by activating the transcriptional coactivator with PDZ-binding motif (TAZ), which activates the TAZ target genes CTGF and Cyr61 [106].

5. Controlling MSC cell behaviour using nanovibrational stimulation

Vibration is classed as an active technique and is viewed as being a cyclically compressive force. Owing to the dynamic nature of the cellular cytoskeleton and its adaptive nature to external stimuli, vibration has been used to investigate mechanotransduction at the cellular level. Experimental apparatus used has ranged from cultureware attached to speakers [107], horizontal vibration [108] and culture plate shakers [109]. A study of periodontal ligament stem cells using low-magnitude, high-frequency vibration (LMHF) over a frequency range of 10 – 180 Hz observed promotion of osteogenic differentiation with the optimal frequency being 50 Hz [109]. A study of MSC combining shear flow (range 0.04 to 5 Pa) and a vibration of magnitudes 0.15g, 1g and 2g with frequencies of 30 Hz and 100 Hz demonstrated a

commitment to an osteogenic lineage [110]. Furthermore, this commitment was due to an up-regulation of the actin remodelling genes including the Wiskott-Aldrich syndrome (WAS). This mechanism was found to be independent of shear flow. Intermittent vibrational loading of hASCs over a 3 day period using a square wave of 50 Hz and 100 Hz with a maximum acceleration magnitude of 3g resulted an increase of some osteogenic markers but failed to approach that of the osteogenic media [107]. In contrast, stimulation of adipose derived stem cells (AT-MSCs) using sub-sonic vibration at a frequency range of 10 – 40 Hz resulted in differentiation of AT-MSCs towards a neural lineage [111]. Due to the diverse range of apparatus utilised in vibrational experiments it is difficult to compare and contrast studies. In addition, it becomes difficult to determine the factor(s) responsible for the observed biological responses when amplitudes are not measured or stated in studies. It may also suggest that differentiation is sensitive to acceleration or that specific frequencies and/or amplitudes play an important role in differentiation driven by mechanotransduction.

It is also of interest that vibration has potential clinical applications, in particular the application of low magnitude, high frequency (LMHF) vibration, also known as whole-body vibration (WBV). These signals have been found to elicit an anabolic effect in murine bone models [112, 113]. WBV has been implicated clinically as being effective in intervention in lower back pain and potential therapeutic effectiveness for sarcopenia and osteoporosis [114]. Safe exposure to WBV is important as long term WBV has been shown to negatively affect certain individuals in industrial settings with lower back pain and sciatica [115].

As a cell interacts with surfaces, nanoscale membrane undulations occur that influence cell-surface interaction [116]. These vibrations produced by the cell have specific amplitudes, frequencies and time scales depending on the cell type [116-122]. Given that cell membranes naturally vibrate at the nanoscale, Curtis and colleagues investigated the application of nanovibrational stimulation using piezo actuators [43]. Osteogenic stimulation of MSCs was noted through activation of the Rho-kinase (ROCK) pathway (**Figure 4**), where significant upregulation of osterix and alkaline phosphatase was measured at day 7 [65]. Osterix is an osteoblast-specific transcription factor and a major effector in bone formation [123]. *In vitro* induction of alkaline phosphate (ALP) has previously been shown as a robust predictor of bone

forming capacity *in vivo*, however, this only occurred when alkaline phosphatase, ALP mRNA levels and ALP activity were observed during *in vitro* osteogenic differentiation [124]. MSCs have demonstrated a strong morphological change in response to nanovibrational stimulation when grown on petri dishes as monolayer, 2D, cultures (**Figure 5**). This occurs due to increased contractility of the cytoskeleton and actin reorganisation. An increase in vinculin, a focal adhesion protein linking integrins to F-actin, is also observed in response to nanovibrational stimulation [125].

Nanovibration induced osteogenesis

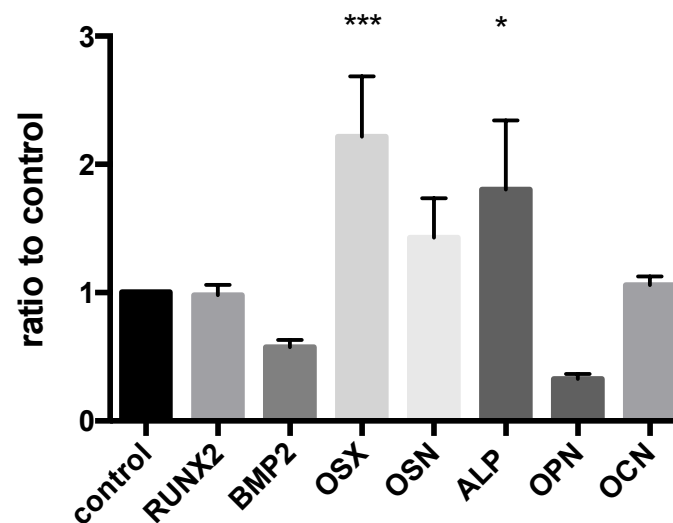


Figure 4: Nanovibrational stimulation induces osteogenesis of MSCs

Osteogenic induction of MSCs by nanovibrational stimulation (1 kHz, 22 nm displacement) after 7 days in 2D culture. Osteogenic gene expression was measured by qRT-PCR for RUNX2 (Runt-related transcription factor 2), BMP2 (bone morphogenetic protein 2), OSX (Osterix), OSN (Osteonectin), ALP (Alkaline phosphatase), OPN (Osteopontin), OCN (Osteocalcin). OSX and ALP were statistically significant higher in the nanostimulated samples in comparison to the unstimulated control by (error bars = SD, 1 patient, n = 4, One-way Anova ***= $p < 0.005$).

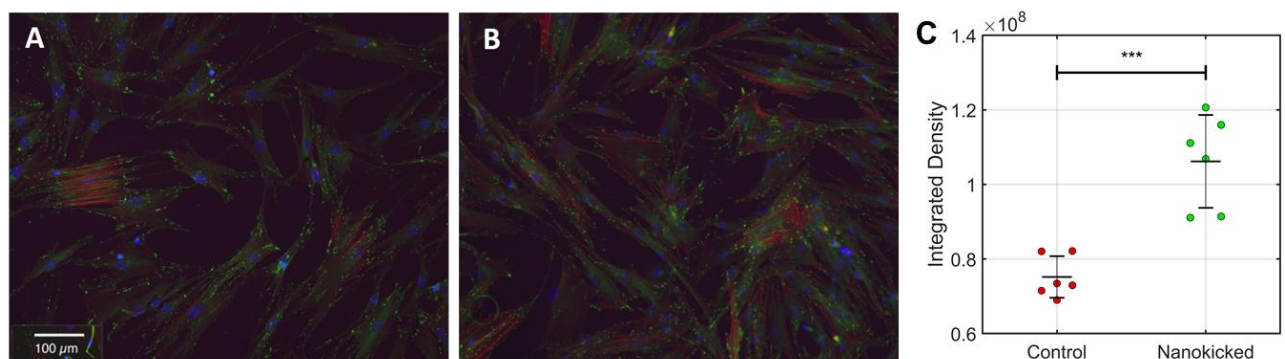


Figure 5: Enhanced adhesion and cytoskeletal remodelling in response to nanovibrational stimulation

Nanovibrational stimulation was applied to MSCs (1 kHz, 22 nm displacement). Fluorescence labelling of actin cytoskeleton (red), vinculin (green) and cell nucleus (blue) was performed. Representative images of (a) control and (b) nanovibrational stimulation applied to MSCs, showing induced remodelling of cell cytoskeleton and increased focal adhesion formation of the stimulated cells. Scale bar = 100 μ m. (C) Integrated density measurements of vinculin expression. Increased vinculin expression in NK (1 kHz) samples in comparison to non-stimulated controls. Data are mean \pm SD, student t-test, $p < 0.001$. $n = 6$.

Nanovibrational stimulation at 500 and 1000 Hz was also shown to influence the size of the nucleus of the cell. The peak force experienced per cell was estimated to be of nanonewtons in magnitude [64]. A combination of nanovibrational stimulation with nanotopographies has also been investigated and showed some benefit of having both environmental and mechanical stimulation, however, nanovibrational stimulation was found to provide a stronger osteoblastic cue [126].

In vitro, cells are routinely cultured in 2D, however, this has been found to be a poor representation of the cellular response *in vivo*, where interactions occur in 3D with extracellular matrix (ECM) alongside a host of bioactive factors [127]. The ECM is composed of macromolecules, glycosaminoglycans and fibrous proteins, of which collagen and fibronectin are components [128]. A study was performed to investigate if nanovibrational stimulation could be applied to a 3D hydrogel structure using the bioreactor discussed in Section 3 [67]. A low elastic modulus scaffold (collagen) was used since high elastic modulus scaffolds alone have been shown to induce osteogenesis of MSCs. FEA modelling and interferometry confirmed transmission of vibration from the nanovibrational bioreactor to the scaffold at the desired 1 kHz frequency. Significant upregulation of BMP2, ALP, OPN, OCN at day 7 and significant upregulation of ALP and collagen I at 14 days was observed (**Figure 6A**). The upregulation of these genes, relative to the control, is strongly indicative of osteogenesis. At day 21, downregulation of RUNX2, ALP, OCN, OPN were observed, which indicated that the transcriptional component of osteogenesis had completed. OCN protein expression at day 21 further confirmed the completion of the transcriptional component of osteogenesis. Mineralisation was confirmed using a combination of von Kossa staining for phosphate, Raman spectroscopy, and X-ray micro-computed tomography. Mineralisation was higher than the control and, at 4 weeks of culture, was higher than that observed in osteoinductive media (**Figure 6B**). Collectively these results provide strong evidence that nanovibrational stimulation

provides a strong osteogenic cue in a non-osteogenic 3D environment. To further elucidate the mechanotransduction mechanisms by which osteogenesis was induced, ion channel sensitivity was tested with the mechanoreceptors: Piezo, TRPV1 and KCNK being differentially expressed in a temporal manner (**Figure 6C**). The involvement of these mechanoreceptors supports a role of intracellular tension in osteogenesis induced mechanotransduction.

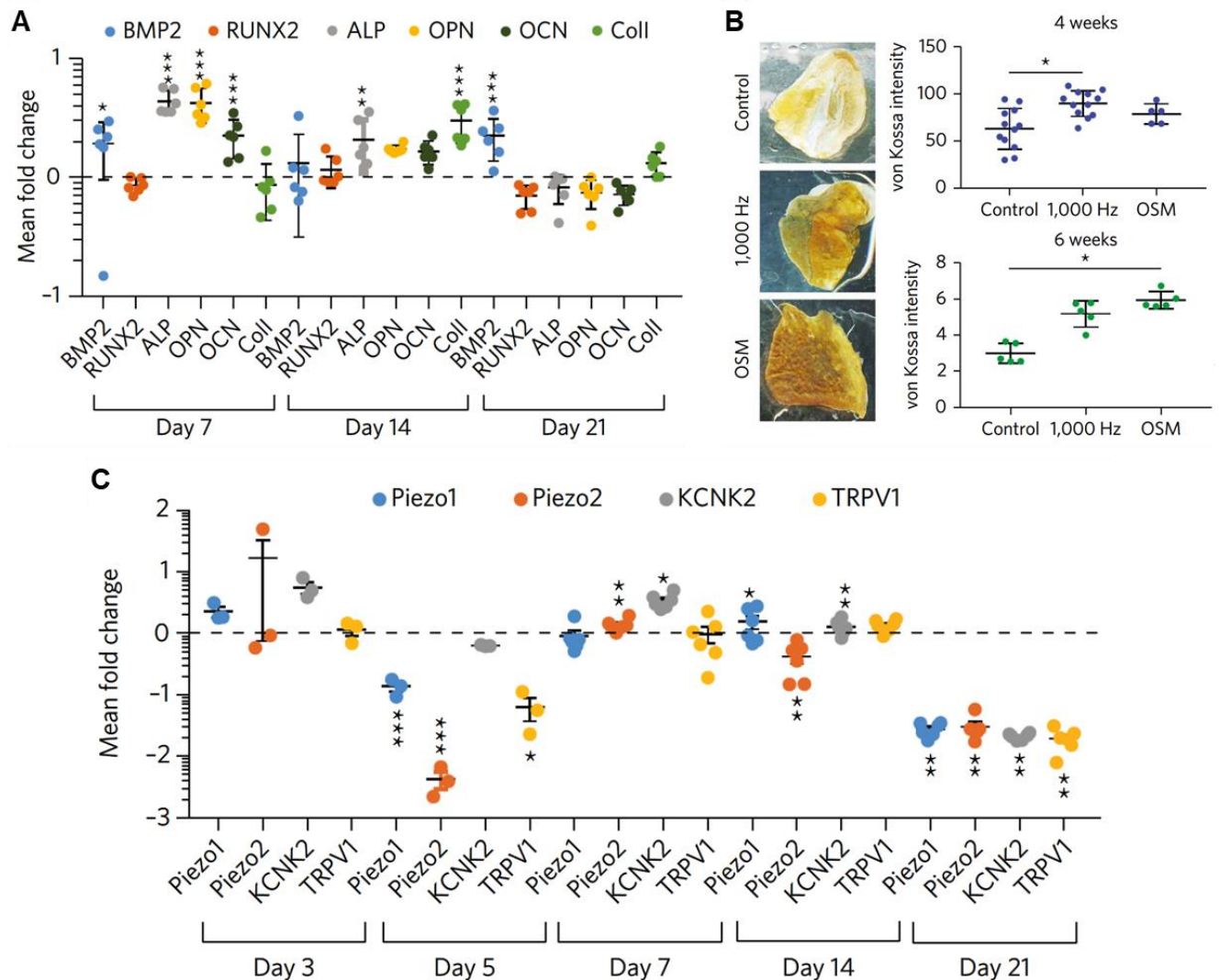


Figure 6: Nanovibrational stimulation induces osteogenesis

(A) qRT-PCR analysis of osteogenic markers over time relative to no vibration. The expression of BMP2, ALP, OPN and OCN was significantly higher in 3D nanovibrated MSCs compared to control. Donors (D) = 1, replicates per donor (r) = 6, technical replicates per replicate (t) = 2; results are mean \pm SD, statistics by Mann–Whitney U-test where *P < 0.05, **P < 0.01 and ***P < 0.001. **(B)** Osteogenesis measurement by Von Kossa staining. Staining was performed after 6 weeks of stimulation at 1 kHz in 3D. Representative gels shown (on the left) were removed from a 6-well plate prior staining. A significantly increased level of staining was observed for the nanostimulated samples relative to the unstimulated controls after 4 weeks (D = 3) (D

= 1 for OSM), $r = 4$), and for nanostimulated samples and osteospecific media (OSM). OSM-treated cells after 6 weeks ($D = 1$, $r = 5$). Results are mean \pm SD, * $P < 0.05$ by ANOVA and Kruskal–Wallis test. **(C)** Temporal qRT–PCR data for Piezo1, Piezo2, TRPV1 and KCNK2 transcripts in MSCs after 3, 5, 7, 14 and 21 days of 3D nanovibrational stimulation displayed as mean fold change with respect to no vibration control cultures. A trend can be observed: high to low expression, followed by recovery, and by another phase of high to low expression, with significant downregulation of all the receptor transcripts at day 21. Donors (D) = 1, replicates per donor (r) = 6 (3 for days 3 and 5), technical replicates per replicate (t) = 2; results are mean \pm SD, statistics by Mann–Whitney U-test, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. Values are displayed in log scale. Figure adapted from Tsimbouri *et al.* [67]

Metabolomics analysis further revealed that TRPV1 is the major contributor to Wnt-mediated osteogenesis, being involved in the activation of Protein kinase C (PKC) and ERK mediated β -catenin activity. A hypothesis for the proposed mechanisms involved in nanovibrational mechanotransduction is summarised schematically (**Figure 7**).

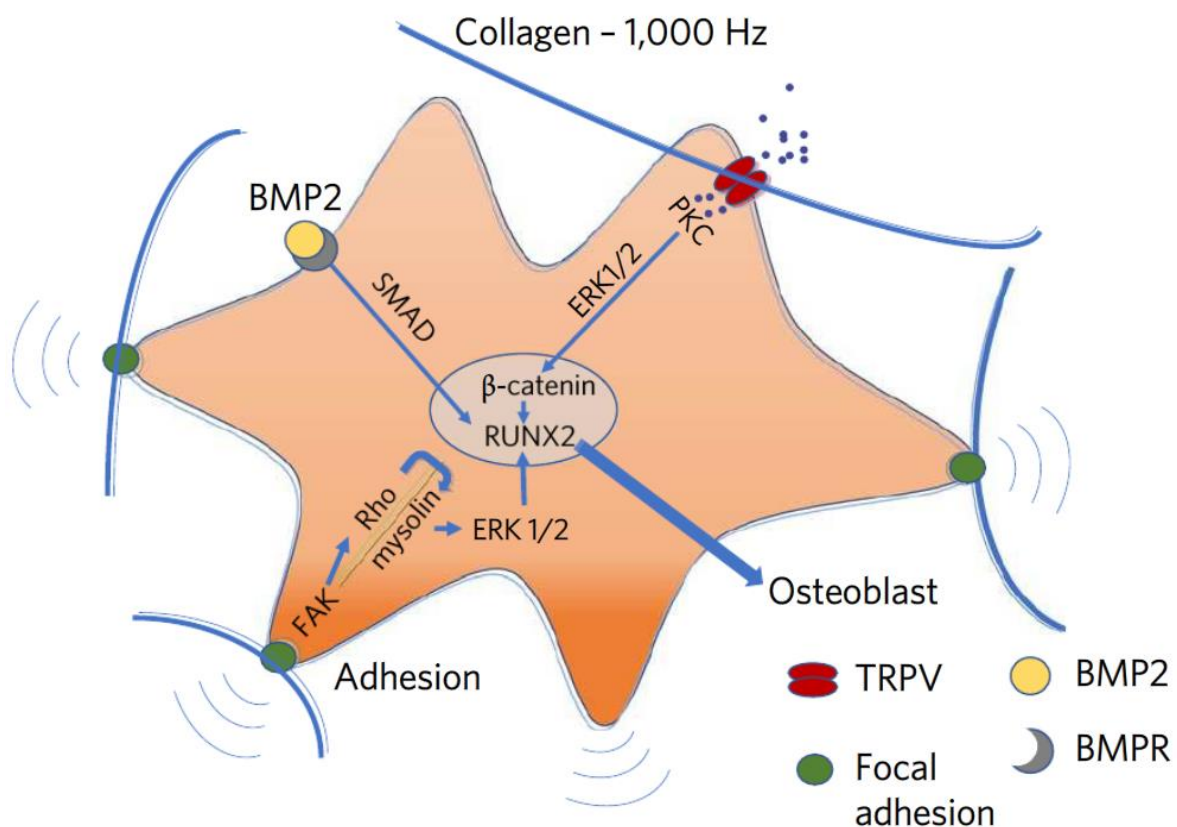


Figure 7: Proposed mechanotransduction mechanism.

Schematic representation of proposed pathways involved in the regulation of osteogenesis in response to nanovibrational stimulation. The TRPV- β -catenin pathway is highlighted as it has been experimentally validated as being important to the MSC nanovibrational induced osteogenesis. Figure adapted from Tsimbouri *et al.* [67].

6. Applying nanovibrational stimulation to prokaryotic cells

Mechanotransduction is deemed essential to the normal function of many mammalian cells, while in contrast this mechanism is rarely considered for bacteria where chemical signals are often regarded as dominant [129-131]. Bacteria, however, routinely experience mechanical forces in flow systems, cell-to-cell interaction, cell-to-surface interaction, twitching motility and the change from planktonic to sessile (biofilm) growth mode. Vibration has been observed in Gram-positive bacteria in a species dependent manner with ranges from 21 nm (*Staphylococcus epidermidis* ATCC35984) up to 145 nm (*Streptococcus salivarius* HB7) [132]. The switch-like model can be applied to bacteria but may suffer the same limitations as found with mammalian cells as bacteria experience a diverse range of mechanical forces. Bacteria have been found experimentally to have mechanosensitive channels, specifically *E. coli* which has 3 classes of mechanosensitive channels in varying size (mscL, mscM, mscS) [133]. There is, however, no direct evidence that these channels respond to external mechanical force due to the limited number of studies of mechanotransduction in bacteria. Evidence of mechanotransduction in bacteria has been shown in *Pseudomonas aeruginosa* (*P. aeruginosa*) where type IV pili (tfp) have been identified as part of a mechanotransduction system, implicated in modulating surface attachment [134]. Retraction of the pilus directly induces signal transduction of a chemotaxis sensory system known as Chp. The Chp system in turn regulates cyclic adenosine monophosphate (cAMP) production and genes associated with virulence. *Neisseria gonorrhoeae* (*N. gonorrhoeae*) tfp have also been demonstrated to play a significant role during infection stimulating microcolony formation and cytoprotection [135, 136]. During infection, *N. gonorrhoeae* tfp and epithelial cells have also been observed to be involved in physical cross-talk and hijacking of the epithelial cell's mechanotransduction mechanism [137].

Other mechanical forces have been demonstrated to influence bacterial phenotype, particularly shear flow. The production of extracellular polymeric substances (EPS) has been shown to increase biofilm cohesion under shear flow [138]. High velocity conditions (tending to and reaching turbulent flow) resulted in thinner biofilms with a greater total amount of polysaccharides and proteins, in addition to decreased attachment compared to biofilms grown under low velocity conditions. External

application of force via vibration to a surface has been performed utilising low energy surface acoustic waves (SAWs) to induce displacements [139]. SAWs were shown to significantly reduce microbial biofilm formation of *Candida albicans*, *E. coli*, *Proteus mirabilis* and *Escherichia faecalis* in Foley catheters. The actuators typically generated vibrations that were in the 100 to 300 kHz range with amplitudes of 300 to 800 nm at source, resulting in SAWs between the 0.2 and 2 nm displacement. Another study has shown that vibration produced by an acoustic method (speaker with Petri dish attached to speaker surface) enhanced biofilm formation of *P. aeruginosa* and *Staphylococcus aureus* (*S. aureus*), using frequencies of 800 and 1600 Hz with displacements of roughly 100 nm [140]. Non-uniform displacement was evidenced by the presence of standing waves in the media, clustering of latex beads and biofilm striation in concentric rings out from the center of the Petri dish, indicators of multiple force vectors.

It is apparent that mechanotransduction is vitally important for microbes to sense and respond to their external environment. Our understanding of microbial mechanotransduction is relatively poor in comparison to that of mammalian cells, yet understanding these mechanisms will provide insight and potential of modulating or controlling bacterial behavior. Nanovibrational stimulation, due to the precision and ability to produce uniform displacements, may prove useful in bacterial mechanotransduction studies. Preliminary studies of the biofilm formation capacity of a number of clinically relevant bacterial species have been performed using the same apparatus utilised in the initial MSC nanokicking study [65]. Vibration with a frequency of 1 kHz and displacement of 30 nm was applied immediately upon inoculation of bacteria over a period of 24 h. Significant reduction in the overall biofilm formation was observed by *P. aeruginosa* PA14, *P. aeruginosa* UWS01, *E. coli* ATCC 35218, *E. coli* JM109 and *S. aureus* NCTC 8178 compared to the control (**Figure 8**) using a total biomass crystal violet staining method. This gives tentative first evidence that nanovibrational stimulation may find application in the control of bacterial biofilm formation of both Gram-positive and Gram-negative bacterial species. It is as yet unknown how bacteria respond at a molecular level to this nanoscale stimulation, research is ongoing to evaluate this response. It may also be of interest to compare the molecular mechanisms involved in bacterial response with those involved with

eukaryotic cellular response, to determine if there is a conserved evolutionary mechanism in the response to mechanical forces.

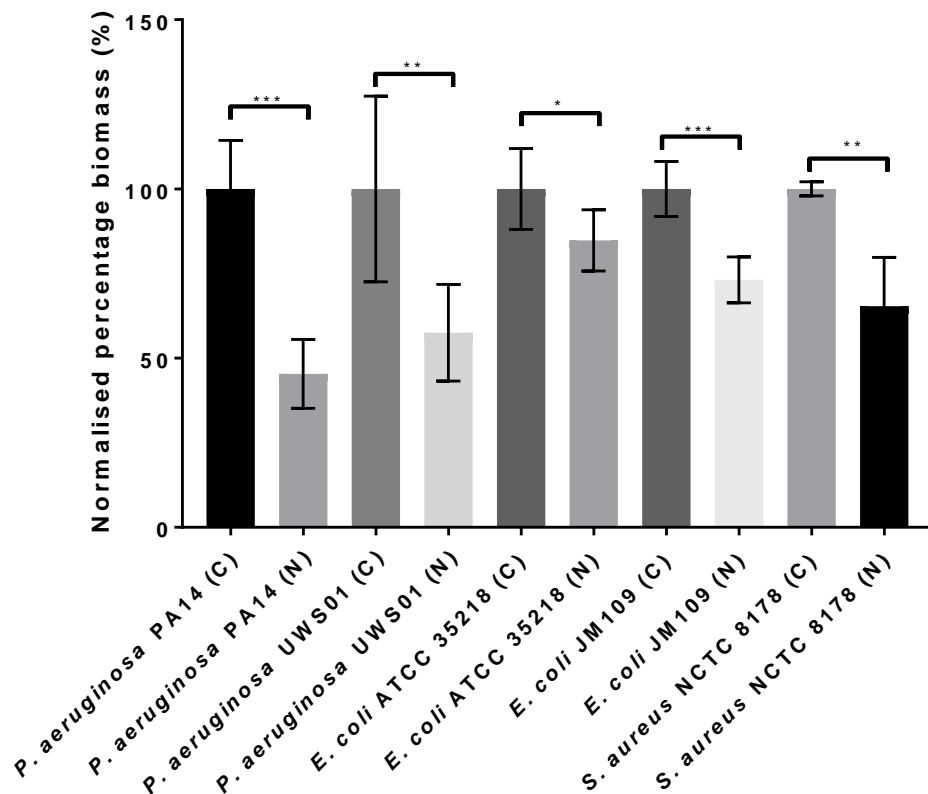


Figure 8: Reduction of bacterial biomass under nanovibrational stimulation

A selection of clinically relevant bacteria were stimulated (1 kHz, 30 nm) for 24 h, using a set-up nominally identical to that used in the initial MSC nanovibrational studies [65]. Crystal violet staining to quantify total biomass was performed at end-point. (C) = Control, (N) = nanovibrational stimulation. Data normalised to control = mean \pm SD, student t-test, * $p < 0.05$, *** $p < 0.001$ *** $p < 0.0001$. $n = 3$.

7. Conclusions

Nanovibrational stimulation of cell cultures can be applied using the novel bioreactor platform described within this paper. This system has been developed through computer simulations (FEA) and validated using laser interferometry, both exploited within the field of gravitational wave astronomy. The bioreactor described in this work has significant advantages compared to previous systems, particularly in relation to the compatibility with standard cultureware, and in the supply of mineralising osteoblasts in a 3D matrix. In addition, as the bioreactor does not come into contact with the mineralising matrix, adaption towards a good manufacturing practice (GMP) process is straightforward and regulatory approval for ancillary reagents is not required.

The role of mechanotransduction and how mechanical signals influence cell behavior is receiving increased attention by researchers and clinicians. The use of nanoscale vibration (nanokicking) has been successfully exploited in promoting osteogenesis from mesenchymal stem cells, both in 2D and 3D constructs. This paves a novel route to fabricate tissue-engineered bone graft for regenerative medicine, for use in the repair of non-union bone fractures. As noted, other cell types, including two clinically relevant forms of bacteria, have been reported to respond to nanoscale vibration, suggesting that the scope of this technique is significantly broader than simply controlling MSC differentiation.

The exploitation of this technique to date has arisen from multidisciplinary research between gravitational wave physicists and biologists. This combination of expertise continues to explore the wider biological potential of this technique in a research and clinical environment as well as developing new technologies.

Competing interests

The authors declare that they have no competing interests

Data accessibility

The raw data is held within the repositories provided in the original publications. New raw data generated for this review is held within

<http://dx.doi.org/10.5525/gla.researchdata.592>

Authors' Contributions

SNR drafted the manuscript, participated in design of bacterial biofilm study and statistical data analysis of study. PC performed interferometry and helped draft the manuscript. FM performed biofilm work and helped in statistical analysis. PGC, FH, MJD & SR helped draft the manuscript. MT performed qRT-PCR gene expression in 2D and 3D nanovibrational stimulation, Von Kossa staining and relevant statistical analysis. HD performed fluorescent microscopy of nanovibrational stimulated BM cells. WGM, CW and SNR conceived biofilm study, participated in study design and statistical data analysis of study. All authors gave final approval for publication.

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References

- [1] Paluch, E. K., Nelson, C. M., Biais, N., Fabry, B., Moeller, J., Pruitt, B. L., Wollnik, C., Kudryasheva, G., Rehfeldt, F. & Federle, W. 2015 Mechanotransduction: use the force(s). *BMC biology* **13**, 47. (DOI:10.1186/s12915-015-0150-4).
- [2] Orr, A. W., Helmke, B. P., Blackman, B. R. & Schwartz, M. A. 2006 Mechanisms of mechanotransduction. *Developmental cell* **10**, 11-20. (DOI:<https://doi.org/10.1016/j.devcel.2005.12.006>).
- [3] Bianchi, L. 2007 Mechanotransduction: touch and feel at the molecular level as modeled in *Caenorhabditis elegans*. *Molecular neurobiology* **36**, 254-271. (DOI:10.1007/s12035-007-8009-5).
- [4] Chalfie, M. 2009 Neurosensory mechanotransduction. *Nature reviews. Molecular cell biology* **10**, 44-52. (DOI:10.1038/nrm2595).
- [5] Hahn, C. & Schwartz, M. A. 2009 Mechanotransduction in vascular physiology and atherogenesis. *Nature reviews. Molecular cell biology* **10**, 53-62. (DOI:10.1038/nrm2596).
- [6] Vollrath, M. A., Kwan, K. Y. & Corey, D. P. 2007 The micromachinery of mechanotransduction in hair cells. *Annual Review of Neuroscience* **30**, 339-365. (DOI:10.1146/annurev.neuro.29.051605.112917).
- [7] Spyropoulou, A., Karamesinis, K. & Basdra, E. K. 2015 Mechanotransduction pathways in bone pathobiology. *Biochimica et biophysica acta* **1852**, 1700-1708. (DOI:10.1016/j.bbadis.2015.05.010).
- [8] Lansman, J. B. & Franco-Obregon, A. 2006 Mechanosensitive ion channels in skeletal muscle: a link in the membrane pathology of muscular dystrophy. *Clinical and experimental pharmacology & physiology* **33**, 649-656. (DOI:10.1111/j.1440-1681.2006.04393.x).
- [9] Chin, L., Xia, Y., Discher, D. E. & Janmey, P. A. 2016 Mechanotransduction in cancer. *Current Opinion in Chemical Engineering* **11**, 77-84. (DOI:<https://doi.org/10.1016/j.coche.2016.01.011>).
- [10] Lin, H. H., Lin, H. K., Lin, I. H., Chiou, Y. W., Chen, H. W., Liu, C. Y., Harn, H. I., Chiu, W. T., Wang, Y. K., Shen, M. R., et al. 2015 Mechanical phenotype of cancer cells: cell softening and loss of stiffness sensing. *Oncotarget* **6**, 20946-20958. (DOI:10.18632/oncotarget.4173).
- [11] Wang, H. B., Dembo, M. & Wang, Y. L. 2000 Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. *American journal of physiology. Cell physiology* **279**, C1345-1350.
- [12] Ibbetson, S. J., Pyne, N. T., Pollard, A. N., Olson, M. F. & Samuel, M. S. 2013 Mechanotransduction pathways promoting tumor progression are activated in invasive human squamous cell carcinoma. *The American Journal of Pathology* **183**, 930-937. (DOI:<https://doi.org/10.1016/j.ajpath.2013.05.014>).
- [13] Duscher, D., Maan, Z. N., Wong, V. W., Rennert, R. C., Januszyk, M., Rodrigues, M., Hu, M., Whitmore, A. J., Whittam, A. J., Longaker, M. T., et al. 2014 Mechanotransduction and fibrosis. *Journal of biomechanics* **47**, 1997-2005. (DOI:10.1016/j.jbiomech.2014.03.031).
- [14] Handorf, A. M., Zhou, Y., Halanski, M. A. & Li, W.-J. 2015 Tissue stiffness dictates development, homeostasis, and disease progression. *Organogenesis* **11**, 1-15. (DOI:10.1080/15476278.2015.1019687).

- [15] Porat, Z., Yaron, I., Katz, B. Z., Kam, Z. & Geiger, B. 2011 Shear flow-induced formation of tubular cell protrusions in multiple myeloma cells. *Journal of cellular physiology* **226**, 3197-3207. (DOI:10.1002/jcp.22680).
- [16] Steward, R. L., Jr., Cheng, C. M., Ye, J. D., Bellin, R. M. & LeDuc, P. R. 2011 Mechanical stretch and shear flow induced reorganization and recruitment of fibronectin in fibroblasts. *Sci Rep* **1**, 147. (DOI:10.1038/srep00147).
- [17] Wang, H., Riha, G. M., Yan, S., Li, M., Chai, H., Yang, H., Yao, Q. & Chen, C. 2005 Shear stress induces endothelial differentiation from a murine embryonic mesenchymal progenitor cell line. *Arteriosclerosis, thrombosis, and vascular biology* **25**, 1817-1823. (DOI:10.1161/01.ATV.0000175840.90510.a8).
- [18] Najrana, T. & Sanchez-Esteban, J. 2016 Mechanotransduction as an adaptation to gravity. *Frontiers in Pediatrics* **4**, 140. (DOI:10.3389/fped.2016.00140).
- [19] Haase, K. & Pelling, A. E. 2015 Investigating cell mechanics with atomic force microscopy. *Journal of the Royal Society Interface* **12**, 20140970. (DOI:10.1098/rsif.2014.0970).
- [20] Honarmandi, P., Lee, H., Lang, M. J. & Kamm, R. D. 2011 A microfluidic system with optical laser tweezers to study mechanotransduction and focal adhesion recruitment. *Lab on a chip* **11**, 684-694. (DOI:10.1039/c0lc00487a).
- [21] Hoffman, B. D., Grashoff, C. & Schwartz, M. A. 2011 Dynamic molecular processes mediate cellular mechanotransduction. *Nature* **475**, 316-323.
- [22] Wolfenson, H., Lavelin, I. & Geiger, B. 2013 Dynamic regulation of the structure and functions of integrin adhesions. *Developmental cell* **24**, 447-458. (DOI:10.1016/j.devcel.2013.02.012).
- [23] Wang, N., Tytell, J. D. & Ingber, D. E. 2009 Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus. *Nature reviews. Molecular cell biology* **10**, 75-82. (DOI:10.1038/nrm2594).
- [24] Ingber, D. 1991 Integrins as mechanochemical transducers. *Current opinion in cell biology* **3**, 841-848.
- [25] Na, S., Collin, O., Chowdhury, F., Tay, B., Ouyang, M., Wang, Y. & Wang, N. 2008 Rapid signal transduction in living cells is a unique feature of mechanotransduction. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 6626-6631. (DOI:10.1073/pnas.0711704105).
- [26] Geiger, B., Spatz, J. P. & Bershadsky, A. D. 2009 Environmental sensing through focal adhesions. *Nature reviews. Molecular cell biology* **10**, 21-33. (DOI:10.1038/nrm2593).
- [27] Sawada, Y., Tamada, M., Dubin-Thaler, B. J., Cherniavskaya, O., Sakai, R., Tanaka, S. & Sheetz, M. P. 2006 Force sensing by mechanical extension of the Src family kinase substrate p130Cas. *Cell* **127**, 1015-1026. (DOI:10.1016/j.cell.2006.09.044).
- [28] Maniotis, A. J., Chen, C. S. & Ingber, D. E. 1997 Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 849-854.
- [29] Wang, N., Naruse, K., Stamenovic, D., Fredberg, J. J., Mijailovich, S. M., Tolic-Norrelykke, I. M., Polte, T., Mannix, R. & Ingber, D. E. 2001 Mechanical behavior in living cells consistent with the tensegrity model. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 7765-7770. (DOI:10.1073/pnas.141199598).

- [30] Homan, S. M., Martinez, R., Benware, A. & LaFlamme, S. E. 2002 Regulation of the association of alpha 6 beta 4 with vimentin intermediate filaments in endothelial cells. *Experimental cell research* **281**, 107-114.
- [31] Sachs, F. 2010 Stretch-activated ion channels: what are they? *Physiology (Bethesda, Md.)* **25**, 50-56. (DOI:10.1152/physiol.00042.2009).
- [32] Haswell, E. S., Phillips, R. & Rees, D. C. 2011 Mechanosensitive channels: what can they do and how do they do it? *Structure (London, England : 1993)* **19**, 1356-1369. (DOI:10.1016/j.str.2011.09.005).
- [33] Martinac, B. 2004 Mechanosensitive ion channels: molecules of mechanotransduction. *Journal of cell science* **117**, 2449-2460. (DOI:10.1242/jcs.01232).
- [34] Ubl, J., Murer, H. & Kolb, H. A. 1988 Ion channels activated by osmotic and mechanical stress in membranes of opossum kidney cells. *The Journal of membrane biology* **104**, 223-232.
- [35] Del Valle, M. E., Cobo, T., Cobo, J. L. & Vega, J. A. 2012 Mechanosensory neurons, cutaneous mechanoreceptors, and putative mechanoproteins. *Microscopy research and technique* **75**, 1033-1043. (DOI:10.1002/jemt.22028).
- [36] Yin, J. & Kuebler, W. M. 2010 Mechanotransduction by TRP channels: general concepts and specific role in the vasculature. *Cell biochemistry and biophysics* **56**, 1-18. (DOI:10.1007/s12013-009-9067-2).
- [37] Sokabe, M., Sachs, F. & Jing, Z. Q. 1991 Quantitative video microscopy of patch clamped membranes stress, strain, capacitance, and stretch channel activation. *Biophys J* **59**, 722-728. (DOI:10.1016/s0006-3495(91)82285-8).
- [38] Martinac, B., Buechner, M., Delcour, A. H., Adler, J. & Kung, C. 1987 Pressure-sensitive ion channel in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America* **84**, 2297-2301.
- [39] Crisp, M., Liu, Q., Roux, K., Rattner, J. B., Shanahan, C., Burke, B., Stahl, P. D. & Hodzic, D. 2006 Coupling of the nucleus and cytoplasm: role of the LINC complex. *The Journal of cell biology* **172**, 41-53. (DOI:10.1083/jcb.200509124).
- [40] Itano, N., Okamoto, S., Zhang, D., Lipton, S. A. & Ruoslahti, E. 2003 Cell spreading controls endoplasmic and nuclear calcium: a physical gene regulation pathway from the cell surface to the nucleus. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 5181-5186. (DOI:10.1073/pnas.0531397100).
- [41] Bode, J., Goetze, S., Heng, H., Krawetz, S. A. & Benham, C. 2003 From DNA structure to gene expression: mediators of nuclear compartmentalization and dynamics. *Chromosome research : an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology* **11**, 435-445.
- [42] Ingber, D. E. 1993 The riddle of morphogenesis: a question of solution chemistry or molecular cell engineering? *Cell* **75**, 1249-1252.
- [43] Curtis, A. S., Reid, S., Martin, I., Vaidyanathan, R., Smith, C. A., Nikukar, H. & Dalby, M. J. 2013 Cell interactions at the nanoscale: piezoelectric stimulation. *IEEE transactions on nanobioscience* **12**, 247-254. (DOI:10.1109/tnb.2013.2257837).
- [44] Ciobanasu, C., Faivre, B. & Le Clainche, C. 2013 Integrating actin dynamics, mechanotransduction and integrin activation: the multiple functions of actin binding proteins in focal adhesions. *European journal of cell biology* **92**, 339-348. (DOI:10.1016/j.ejcb.2013.10.009).
- [45] Rosenberg, N. 2003 The role of the cytoskeleton in mechanotransduction in human osteoblast-like cells. *Human & experimental toxicology* **22**, 271-274. (DOI:10.1191/0960327103ht362oa).

- [46] Kerr, J. P., Robison, P., Shi, G., Bogush, A. I., Kempema, A. M., Hexum, J. K., Becerra, N., Harki, D. A., Martin, S. S., Raiteri, R., et al. 2015 Detyrosinated microtubules modulate mechanotransduction in heart and skeletal muscle. *Nature Communications* **6**, 8526. (DOI:10.1038/ncomms9526 <https://www.nature.com/articles/ncomms9526#supplementary-information>).
- [47] Sanghvi-Shah, R. & Weber, G. F. 2017 Intermediate filaments at the junction of mechanotransduction, migration, and development. *Frontiers in Cell and Developmental Biology* **5**, 81. (DOI:10.3389/fcell.2017.00081).
- [48] Lang, T., LeBlanc, A., Evans, H., Lu, Y., Genant, H. & Yu, A. 2004 Cortical and trabecular bone mineral loss from the spine and hip in long-duration spaceflight. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* **19**, 1006-1012. (DOI:10.1359/jbmr.040307).
- [49] Nabavi, N., Khandani, A., Camirand, A. & Harrison, R. E. 2011 Effects of microgravity on osteoclast bone resorption and osteoblast cytoskeletal organization and adhesion. *Bone* **49**, 965-974. (DOI:10.1016/j.bone.2011.07.036).
- [50] Cui, Y., Hameed, F. M., Yang, B., Lee, K., Pan, C. Q., Park, S. & Sheetz, M. 2015 Cyclic stretching of soft substrates induces spreading and growth. *Nature Communications* **6**, 6333. (DOI:10.1038/ncomms7333).
- [51] Matsugaki, A., Fujiwara, N. & Nakano, T. 2013 Continuous cyclic stretch induces osteoblast alignment and formation of anisotropic collagen fiber matrix. *Acta Biomaterialia* **9**, 7227-7235. (DOI:<https://doi.org/10.1016/j.actbio.2013.03.015>).
- [52] Gillespie, P. G. & Müller, U. 2009 Mechanotransduction by hair cells: models, molecules, and mechanisms. *Cell* **139**, 33-44. (DOI:10.1016/j.cell.2009.09.010).
- [53] Krishnan, R., Park, C. Y., Lin, Y. C., Mead, J., Jaspers, R. T., Trepatt, X., Lenormand, G., Tambe, D., Smolensky, A. V., Knoll, A. H., et al. 2009 Reinforcement versus fluidization in cytoskeletal mechanoresponsiveness. *PloS one* **4**, e5486. (DOI:10.1371/journal.pone.0005486).
- [54] Tsimbouri, P. M., McMurray, R. J., Burgess, K. V., Alakpa, E. V., Reynolds, P. M., Murawski, K., Kingham, E., Oreffo, R. O., Gadegaard, N. & Dalby, M. J. 2012 Using nanotopography and metabolomics to identify biochemical effectors of multipotency. *ACS Nano* **6**, 10239-10249.
- [55] Engler, A. J., Sen, S., Sweeney, H. L. & Discher, D. E. 2006 Matrix elasticity directs stem cell lineage specification. *Cell* **126**, 677-689. (DOI:10.1016/j.cell.2006.06.044).
- [56] McBeath, R., Pirone, D. M., Nelson, C. M., Bhadriraju, K. & Chen, C. S. 2004 Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Developmental cell* **6**, 483-495.
- [57] Mack, P. J., Kaazempur-Mofrad, M. R., Karcher, H., Lee, R. T. & Kamm, R. D. 2004 Force-induced focal adhesion translocation: effects of force amplitude and frequency. *American Journal of Physiology - Cell Physiology* **287**, C954-C962. (DOI:10.1152/ajpcell.00567.2003).
- [58] Shafrir, Y. & Forgacs, G. 2002 Mechanotransduction through the cytoskeleton. *American Journal of Physiology - Cell Physiology* **282**, C479-C486. (DOI:10.1152/ajpcell.00394.2001).
- [59] Hu, S. & Wang, N. 2006 Control of stress propagation in the cytoplasm by prestress and loading frequency. *Molecular & cellular biomechanics : MCB* **3**, 49-60.
- [60] Ricci, A. J., Kennedy, H. J., Crawford, A. C. & Fettiplace, R. 2005 The transduction channel filter in auditory hair cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **25**, 7831-7839. (DOI:10.1523/jneurosci.1127-05.2005).

- [61] Deguchi, S., Ohashi, T. & Sato, M. 2006 Tensile properties of single stress fibers isolated from cultured vascular smooth muscle cells. *J Biomech* **39**, 2603-2610. (DOI:10.1016/j.jbiomech.2005.08.026).
- [62] Tsuda, Y., Yasutake, H., Ishijima, A. & Yanagida, T. 1996 Torsional rigidity of single actin filaments and actin-actin bond breaking force under torsion measured directly by *in vitro* micromanipulation. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 12937-12942.
- [63] del Rio, A., Perez-Jimenez, R., Liu, R., Roca-Cusachs, P., Fernandez, J. M. & Sheetz, M. P. 2009 Stretching single talin rod molecules activates vinculin binding. *Science (New York, N.Y.)* **323**, 638-641. (DOI:10.1126/science.1162912).
- [64] Nikukar, H., Childs, P. G., Curtis, A. S. G., Martin, I. W., Riehle, M. O., Dalby, M. J. & Reid, S. 2016 Production of nanoscale vibration for stimulation of human mesenchymal stem cells. *Journal of Biomedical Nanotechnology* **12**, 1478-1488. (DOI:10.1166/jbn.2016.2264).
- [65] Nikukar, H., Reid, S., Tsimbouri, P. M., Riehle, M. O., Curtis, A. S. G. & Dalby, M. J. 2013 Osteogenesis of mesenchymal stem cells by nanoscale mechanotransduction. *ACS Nano* **7**, 2758-2767. (DOI:10.1021/nn400202j).
- [66] Childs, P. G., Reid, S., Dalby, M. J., Pemberton, G. D., Nikukar, H. & Curtis, A. S. G. 2017 Mechanical Bioreactor. *PCT/EP2016/06970*.
- [67] Tsimbouri, P. M., Childs, P. G., Pemberton, G. D., Yang, J., Jayawarna, V., Orapiriyakul, W., Burgess, K., González-García, C., Blackburn, G., Thomas, D., et al. 2017 Stimulation of 3D osteogenesis by mesenchymal stem cells using a nanovibrational bioreactor. *Nature Biomedical Engineering* **1**, 758-770. (DOI:10.1038/s41551-017-0127-4).
- [68] Abbott, B. P. & Abbott, R. & Adhikari, R. & Ajith, P. & Allen, B. & Allen, G. & Amin, R. S. & Anderson, S. B. & Anderson, W. G. & Arain, M. A., et al. 2009 LIGO: the laser Interferometer gravitational-wave observatory. *Reports on Progress in Physics* **72**, 076901.
- [69] Collaboration, L. S. & Virgo, C. & Abbott, B. P. & Abbott, R. & Abbott, T. D. & Abernathy, M. R. & Acernese, F. & Ackley, K. & Adams, C. & Adams, T., et al. 2016 Observation of gravitational waves from a binary black hole merger. *Physical Review Letters* **116**, 061102.
- [70] Collaboration, L. S. & Virgo, C. & Abbott, B. P. & Abbott, R. & Abbott, T. D. & Acernese, F. & Ackley, K. & Adams, C. & Adams, T. & Addesso, P., et al. 2017 GW170817: observation of gravitational waves from a binary neutron star inspiral. *Physical Review Letters* **119**, 161101.
- [71] Pitkin, M., Reid, S., Rowan, S. & Hough, J. 2011 Gravitational wave detection by interferometry (ground and space). *Living Reviews in Relativity* **14**, 5. (DOI:10.12942/lrr-2011-5).
- [72] Robinson, B. T., Metcalfe, D., Cuff, A. V., Pidgeon, T. E., Hewitt, K. J., Gibbs, V. N., Rossiter, D. J. & Griffin, X. L. 2015 Surgical techniques for autologous bone harvesting from the iliac crest in adults. *Cochrane Database of Systematic Reviews*. (DOI:10.1002/14651858.CD011783).
- [73] Dimitriou, R., Mataliotakis, G. I., Angoules, A. G., Kanakaris, N. K. & Giannoudis, P. V. 2011 Complications following autologous bone graft harvesting from the iliac crest and using the RIA: a systematic review. *Injury* **42 Suppl 2**, S3-15. (DOI:10.1016/j.injury.2011.06.015).
- [74] Garnero, P., Sornay-Rendu, E., Chapuy, M. C. & Delmas, P. D. 1996 Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis.

Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research **11**, 337-349. (DOI:10.1002/jbmr.5650110307).

[75] Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R., Mosca, J. D., Moorman, M. A., Simonetti, D. W., Craig, S. & Marshak, D. R. 1999 Multilineage potential of adult human mesenchymal stem cells. *Science (New York, N.Y.)* **284**, 143-147.

[76] Crisan, M., Yap, S., Casteilla, L., Chen, C. W., Corselli, M., Park, T. S., Andriolo, G., Sun, B., Zheng, B., Zhang, L., et al. 2008 A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell stem cell* **3**, 301-313. (DOI:10.1016/j.stem.2008.07.003).

[77] da Silva Meirelles, L., Chagastelles, P. C. & Nardi, N. B. 2006 Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *Journal of cell science* **119**, 2204-2213. (DOI:10.1242/jcs.02932).

[78] Ramakrishnan, A., Torok-Storb, B. & Pillai, M. M. 2013 Primary marrow derived stromal cells: isolation and manipulation. *Methods in molecular biology (Clifton, N.J.)* **1035**, 75-101. (DOI:10.1007/978-1-62703-508-8_8).

[79] Tsuji, W., Rubin, J. P. & Marra, K. G. 2014 Adipose-derived stem cells: Implications in tissue regeneration. *World Journal of Stem Cells* **6**, 312-321. (DOI:10.4252/wjsc.v6.i3.312).

[80] Chen, C. S. 2008 Mechanotransduction - a field pulling together? *Journal of cell science* **121**, 3285-3292. (DOI:10.1242/jcs.023507).

[81] Boyan, B. D., Hummert, T. W., Dean, D. D. & Schwartz, Z. 1996 Role of material surfaces in regulating bone and cartilage cell response. *Biomaterials* **17**, 137-146.

[82] Curtis, A. & Wilkinson, C. 1997 Topographical control of cells. *Biomaterials* **18**, 1573-1583.

[83] Ito, Y. 1999 Surface micropatterning to regulate cell functions. *Biomaterials* **20**, 2333-2342. (DOI:[https://doi.org/10.1016/S0142-9612\(99\)00162-3](https://doi.org/10.1016/S0142-9612(99)00162-3)).

[84] Singhvi, R., Stephanopoulos, G. & Wang, D. I. 1994 Effects of substratum morphology on cell physiology. *Biotechnology and bioengineering* **43**, 764-771. (DOI:10.1002/bit.260430811).

[85] du Roure, O., Saez, A., Buguin, A., Austin, R. H., Chavrier, P., Siberzan, P. & Ladoux, B. 2005 Force mapping in epithelial cell migration. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 2390-2395. (DOI:10.1073/pnas.0408482102).

[86] Fu, J., Wang, Y.-K., Yang, M. T., Desai, R. A., Yu, X., Liu, Z. & Chen, C. S. 2010 Mechanical regulation of cell function with geometrically modulated elastomeric substrates. *Nature Methods* **7**, 733. (DOI:10.1038/nmeth.1487 <https://www.nature.com/articles/nmeth.1487#supplementary-information>).

[87] Wan, Y., Wang, Y., Liu, Z., Qu, X., Han, B., Bei, J. & Wang, S. 2005 Adhesion and proliferation of OCT-1 osteoblast-like cells on micro- and nano-scale topography structured poly(l-lactide). *Biomaterials* **26**, 4453-4459. (DOI:<https://doi.org/10.1016/j.biomaterials.2004.11.016>).

[88] Lu, X. & Leng, Y. 2009 Comparison of the osteoblast and myoblast behavior on hydroxyapatite microgrooves. *Journal of biomedical materials research. Part B, Applied biomaterials* **90**, 438-445. (DOI:10.1002/jbm.b.31304).

[89] Oakley, C., Jaeger, N. A. & Brunette, D. M. 1997 Sensitivity of fibroblasts and their cytoskeletons to substratum topographies: topographic guidance and topographic compensation by micromachined grooves of different dimensions. *Experimental cell research* **234**, 413-424. (DOI:10.1006/excr.1997.3625).

- [90] Hayes, J. S., Khan, I. M., Archer, C. W. & Richards, R. G. 2010 The role of surface microtopography in the modulation of osteoblast differentiation. *European cells & materials* **20**, 98-108.
- [91] Masaki, C., Schneider, G. B., Zaharias, R., Seabold, D. & Stanford, C. 2005 Effects of implant surface microtopography on osteoblast gene expression. *Clinical oral implants research* **16**, 650-656. (DOI:10.1111/j.1600-0501.2005.01170.x).
- [92] Dalby, M. J., Gadegaard, N., Tare, R., Andar, A., Riehle, M. O., Herzyk, P., Wilkinson, C. D. W. & Oreffo, R. O. C. 2007 The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. *Nature Materials* **6**, 997. (DOI:10.1038/nmat2013
<https://www.nature.com/articles/nmat2013#supplementary-information>).
- [93] Kulangara, K., Yang, Y., Yang, J. & Leong, K. W. 2012 Nanotopography as modulator of human mesenchymal stem cell function. *Biomaterials* **33**, 4998-5003. (DOI:10.1016/j.biomaterials.2012.03.053).
- [94] McNamara, L. E., Sjostrom, T., Seunarine, K., Meek, R. D., Su, B. & Dalby, M. J. 2014 Investigation of the limits of nanoscale filopodial interactions. *Journal of tissue engineering* **5**, 2041731414536177. (DOI:10.1177/2041731414536177).
- [95] Tsimbouri, P., Gadegaard, N., Burgess, K., White, K., Reynolds, P., Herzyk, P., Oreffo, R. & Dalby, M. J. 2014 Nanotopographical effects on mesenchymal stem cell morphology and phenotype. *Journal of cellular biochemistry* **115**, 380-390. (DOI:10.1002/jcb.24673).
- [96] Dalby, M. J., Riehle, M. O., Johnstone, H. J., Affrossman, S. & Curtis, A. S. 2002 Polymer-demixed nanotopography: control of fibroblast spreading and proliferation. *Tissue engineering* **8**, 1099-1108. (DOI:10.1089/107632702320934191).
- [97] Kilian, K. A., Bugarija, B., Lahn, B. T. & Mrksich, M. 2010 Geometric cues for directing the differentiation of mesenchymal stem cells. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 4872-4877. (DOI:10.1073/pnas.0903269107).
- [98] Billiet, T., Vandenhaute, M., Schelfhout, J., Van Vlierberghe, S. & Dubrue, P. 2012 A review of trends and limitations in hydrogel-rapid prototyping for tissue engineering. *Biomaterials* **33**, 6020-6041. (DOI:<https://doi.org/10.1016/j.biomaterials.2012.04.050>).
- [99] Drury, J. L. & Mooney, D. J. 2003 Hydrogels for tissue engineering: scaffold design variables and applications. *Biomaterials* **24**, 4337-4351. (DOI:[https://doi.org/10.1016/S0142-9612\(03\)00340-5](https://doi.org/10.1016/S0142-9612(03)00340-5)).
- [100] Geckil, H., Xu, F., Zhang, X., Moon, S. & Demirci, U. 2010 Engineering hydrogels as extracellular matrix mimics. *Nanomedicine (London, England)* **5**, 469-484. (DOI:10.2217/nnm.10.12).
- [101] Green, J. J. & Elisseeff, J. H. 2016 Mimicking biological functionality with polymers for biomedical applications. *Nature* **540**, 386. (DOI:10.1038/nature21005).
- [102] Vincent, L. G., Choi, Y. S., Alonso-Latorre, B., del Alamo, J. C. & Engler, A. J. 2013 Mesenchymal stem cell durotaxis depends on substrate stiffness gradient strength. *Biotechnology journal* **8**, 472-484. (DOI:10.1002/biot.201200205).
- [103] Marie, P. J. 2008 Transcription factors controlling osteoblastogenesis. *Archives of biochemistry and biophysics* **473**, 98-105. (DOI:10.1016/j.abb.2008.02.030).
- [104] Huang, C. Y., Hagar, K. L., Frost, L. E., Sun, Y. & Cheung, H. S. 2004 Effects of cyclic compressive loading on chondrogenesis of rabbit bone-marrow derived mesenchymal stem cells. *Stem cells (Dayton, Ohio)* **22**, 313-323. (DOI:10.1634/stemcells.22-3-313).

- [105] Shyy, J. Y. & Chien, S. 2002 Role of integrins in endothelial mechanosensing of shear stress. *Circulation research* **91**, 769-775.
- [106] Kim, K. M., Choi, Y. J., Hwang, J.-H., Kim, A. R., Cho, H. J., Hwang, E. S., Park, J. Y., Lee, S.-H. & Hong, J.-H. 2014 Shear stress induced by an interstitial level of slow flow Increases the osteogenic differentiation of mesenchymal stem cells through TAZ activation. *PloS one* **9**, e92427. (DOI:10.1371/journal.pone.0092427).
- [107] Tirkkonen, L., Halonen, H., Hyttinen, J., Kuokkanen, H., Sievanen, H., Koivisto, A. M., Mannerstrom, B., Sandor, G. K., Suuronen, R., Miettinen, S., et al. 2011 The effects of vibration loading on adipose stem cell number, viability and differentiation towards bone-forming cells. *Journal of the Royal Society, Interface* **8**, 1736-1747. (DOI:10.1098/rsif.2011.0211).
- [108] Gaston, J., Quinchia Rios, B., Bartlett, R., Berchtold, C. & Thibeault, S. L. 2012 The response of vocal fold fibroblasts and mesenchymal stromal cells to vibration. *PloS one* **7**, e30965. (DOI:10.1371/journal.pone.0030965).
- [109] Zhang, C., Li, J., Zhang, L., Zhou, Y., Hou, W., Quan, H., Li, X., Chen, Y. & Yu, H. 2012 Effects of mechanical vibration on proliferation and osteogenic differentiation of human periodontal ligament stem cells. *Archives of oral biology* **57**, 1395-1407. (DOI:10.1016/j.archoralbio.2012.04.010).
- [110] Uzer, G., Pongkitwitoon, S., Ete Chan, M. & Judex, S. 2013 Vibration induced osteogenic commitment of mesenchymal stem cells is enhanced by cytoskeletal remodeling but not fluid shear. *J Biomech* **46**, 2296-2302. (DOI:10.1016/j.jbiomech.2013.06.008).
- [111] Choi, Y.-K., Cho, H., Seo, Y.-K., Yoon, H.-H. & Park, J.-K. 2012 Stimulation of sub-sonic vibration promotes the differentiation of adipose tissue-derived mesenchymal stem cells into neural cells. *Life Sciences* **91**, 329-337. (DOI:<https://doi.org/10.1016/j.lfs.2012.07.022>).
- [112] Garman, R., Gaudette, G., Donahue, L. R., Rubin, C. & Judex, S. 2007 Low-level accelerations applied in the absence of weight bearing can enhance trabecular bone formation. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society* **25**, 732-740. (DOI:10.1002/jor.20354).
- [113] Xie, L., Jacobson, J. M., Choi, E. S., Busa, B., Donahue, L. R., Miller, L. M., Rubin, C. T. & Judex, S. 2006 Low-level mechanical vibrations can influence bone resorption and bone formation in the growing skeleton. *Bone* **39**, 1059-1066. (DOI:10.1016/j.bone.2006.05.012).
- [114] Dionello, C. F., Sá-Caputo, D., Pereira, H., Sousa-Gonçalves, C. R., Maiworm, A. I., Morel, D. S., Moreira-Marconi, E., Paineiras-Domingos, L. L., Bemben, D. & Bernardo-Filho, M. 2016 Effects of whole body vibration exercises on bone mineral density of women with postmenopausal osteoporosis without medications: novel findings and literature review. *Journal of Musculoskeletal & Neuronal Interactions* **16**, 193-203.
- [115] Burström, L., Nilsson, T. & Wahlström, J. 2015 Whole-body vibration and the risk of low back pain and sciatica: a systematic review and meta-analysis. *International Archives of Occupational and Environmental Health* **88**, 403-418. (DOI:10.1007/s00420-014-0971-4).
- [116] Pierres, A., Benoliel, A.-M., Touchard, D. & Bongrand, P. 2008 How cells tiptoe on adhesive surfaces before sticking. *Biophysical Journal* **94**, 4114-4122. (DOI:10.1529/biophysj.107.125278).
- [117] Szabo, B., Selmeczi, D., Kornyei, Z., Madarasz, E. & Rozlosnik, N. 2002 Atomic force microscopy of height fluctuations of fibroblast cells. *Physical review. E*,

- Statistical, nonlinear, and soft matter physics* **65**, 041910. (DOI:10.1103/PhysRevE.65.041910).
- [118] Shamri, R., Grabovsky, V., Gauguier, J. M., Feigelson, S., Manevich, E., Kolanus, W., Robinson, M. K., Staunton, D. E., von Andrian, U. H. & Alon, R. 2005 Lymphocyte arrest requires instantaneous induction of an extended LFA-1 conformation mediated by endothelium-bound chemokines. *Nature immunology* **6**, 497-506. (DOI:10.1038/ni1194).
- [119] Rappaz, B., Barbul, A., Hoffmann, A., Boss, D., Korenstein, R., Depeursinge, C., Magistretti, P. J. & Marquet, P. 2009 Spatial analysis of erythrocyte membrane fluctuations by digital holographic microscopy. *Blood cells, molecules & diseases* **42**, 228-232. (DOI:10.1016/j.bcmd.2009.01.018).
- [120] Krol, A., Grinfeldt, M. G., Levin, S. V. & Smilgavichus, A. D. 1990 Local mechanical oscillations of the cell surface within the range 0.2-30 Hz. *European biophysics journal : EBJ* **19**, 93-99.
- [121] Evans, J., Gratzner, W., Mohandas, N., Parker, K. & Sleep, J. 2008 Fluctuations of the red blood cell membrane: relation to mechanical properties and lack of ATP dependence. *Biophysical Journal* **94**, 4134-4144. (DOI:<https://doi.org/10.1529/biophysj.107.117952>).
- [122] Evans, E. 1989 Kinetics of granulocyte phagocytosis: rate limited by cytoplasmic viscosity and constrained by cell size. *Cell motility and the cytoskeleton* **14**, 544-551. (DOI:10.1002/cm.970140411).
- [123] Sinha, K. M. & Zhou, X. 2013 Genetic and molecular control of Osterix in skeletal formation. *Journal of cellular biochemistry* **114**, 975-984. (DOI:10.1002/jcb.24439).
- [124] Prins, H.-J., Braat, A. K., Gawlitta, D., Dhert, W. J. A., Egan, D. A., Tijssen-Slump, E., Yuan, H., Coffey, P. J., Rozemuller, H. & Martens, A. C. 2014 *In vitro* induction of alkaline phosphatase levels predicts *in vivo* bone forming capacity of human bone marrow stromal cells. *Stem Cell Research* **12**, 428-440. (DOI:<https://doi.org/10.1016/j.scr.2013.12.001>).
- [125] Atherton, P., Stutchbury, B., Jethwa, D. & Ballestrem, C. 2016 Mechanosensitive components of integrin adhesions: Role of vinculin. *Experimental cell research* **343**, 21-27. (DOI:10.1016/j.yexcr.2015.11.017).
- [126] Pemberton, G. D., Childs, P., Reid, S., Nikukar, H., Tsimbouri, P. M., Gadegaard, N., Curtis, A. S. & Dalby, M. J. 2015 Nanoscale stimulation of osteoblastogenesis from mesenchymal stem cells: nanotopography and nanokicking. *Nanomedicine (London, England)* **10**, 547-560. (DOI:10.2217/nnm.14.134).
- [127] Edmondson, R., Broglie, J. J., Adcock, A. F. & Yang, L. 2014 Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. *Assay and Drug Development Technologies* **12**, 207-218. (DOI:10.1089/adt.2014.573).
- [128] Frantz, C., Stewart, K. M. & Weaver, V. M. 2010 The extracellular matrix at a glance. *Journal of cell science* **123**, 4195-4200. (DOI:10.1242/jcs.023820).
- [129] and, M. B. M. & Bassler, B. L. 2001 Quorum sensing in bacteria. *Annual Review of Microbiology* **55**, 165-199. (DOI:10.1146/annurev.micro.55.1.165).
- [130] Papenfort, K. & Bassler, B. L. 2016 Quorum sensing signal-response systems in Gram-negative bacteria. *Nature Reviews Microbiology* **14**, 576. (DOI:10.1038/nrmicro.2016.89).
- [131] Whiteley, M., Diggle, S. P. & Greenberg, E. P. 2017 Progress in and promise of bacterial quorum sensing research. *Nature* **551**, 313. (DOI:10.1038/nature24624).
- [132] Song, L., Sjollem, J., Sharma, P. K., Kaper, H. J., van der Mei, H. C. & Busscher, H. J. 2014 Nanoscopic vibrations of bacteria with different cell-wall

- properties adhering to surfaces under flow and static conditions. *ACS Nano* **8**, 8457-8467. (DOI:10.1021/nn5030253).
- [133] Edwards, M. D., Black, S., Rasmussen, T., Rasmussen, A., Stokes, N. R., Stephen, T.-L., Miller, S. & Booth, I. R. 2012 Characterization of three novel mechanosensitive channel activities in *Escherichia coli*. *Channels* **6**, 272-281. (DOI:10.4161/chan.20998).
- [134] Persat, A., Inclan, Y. F., Engel, J. N., Stone, H. A. & Gitai, Z. 2015 Type IV pili mechanochemically regulate virulence factors in *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences of the United States of America* **112**, 7563-7568. (DOI:10.1073/pnas.1502025112).
- [135] Higashi, D. L., Lee, S. W., Snyder, A., Weyand, N. J., Bakke, A. & So, M. 2007 Dynamics of *Neisseria gonorrhoeae* attachment: microcolony development, cortical plaque formation, and cytoprotection. *Infection and Immunity* **75**, 4743-4753. (DOI:10.1128/iai.00687-07).
- [136] Howie, H. L., Glogauer, M. & So, M. 2005 The *N. gonorrhoeae* type IV pilus stimulates mechanosensitive pathways and cytoprotection through a pilT-dependent mechanism. *PLOS Biology* **3**, e100. (DOI:10.1371/journal.pbio.0030100).
- [137] Lee, S. W., Higashi, D. L., Snyder, A., Merz, A. J., Potter, L. & So, M. 2005 PilT is required for PI(3,4,5)P3-mediated crosstalk between *Neisseria gonorrhoeae* and epithelial cells. *Cellular microbiology* **7**, 1271-1284. (DOI:10.1111/j.1462-5822.2005.00551.x).
- [138] Wang, C., Miao, L., Hou, J., Wang, P., Qian, J. & Dai, S. 2014 The effect of flow velocity on the distribution and composition of extracellular polymeric substances in biofilms and the detachment mechanism of biofilms. *Water science and technology : a journal of the International Association on Water Pollution Research* **69**, 825-832. (DOI:10.2166/wst.2013.785).
- [139] Hazan, Z., Zumeris, J., Jacob, H., Raskin, H., Kratysh, G., Vishnia, M., Dror, N., Barliya, T., Mandel, M. & Lavie, G. 2006 Effective prevention of microbial biofilm formation on medical devices by low-energy surface acoustic waves. *Antimicrobial Agents and Chemotherapy* **50**, 4144-4152. (DOI:10.1128/AAC.00418-06).
- [140] Murphy, M. F., Edwards, T., Hobbs, G., Shepherd, J. & Bezombes, F. 2016 Acoustic vibration can enhance bacterial biofilm formation. *Journal of bioscience and bioengineering* **122**, 765-770. (DOI:10.1016/j.jbiosc.2016.05.010).